Regional Operational Plan CF.5J.2014.02

A Pilot Study Investigating the Applicability of a Novel Direct Aging Technique to Commercially Important Crustaceans in Alaska

by

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Quinn Smith,
and
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July 2014

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat or long
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$log_{2,}$ etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

REGIONAL OPERATIONAL PLAN CF.5J.2014.02

A PILOT STUDY INVESTIGATING THE APPLICABILITY OF A NOVEL DIRECT AGING TECHNIQUE TO COMMERCIALLY IMPORTANT CRUSTACEANS IN ALASKA

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Alaska Department of Fish and Game
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July 2014

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Signature Page

Project Title: A pilot study investigating the applicability of a novel direct aging technique to commercially important crustaceans in Alaska

Project leader(s): Joel Webb, Laura Stichert, Quinn Smith, and Kevin McNeel

Division, Region, and Area:

Commercial Fisheries, Headquarters, Juneau (Webb)
Commercial Fisheries, Westward Region, Kodiak (Stichert)
Commercial Fisheries, Southeast Region, Douglas (Smith)
Commercial Fisheries, Age Determination Unit, Juneau (McNeel)

Project Nomenclature:
Southeast Alaska Shrimp Research, Bering Sea Crab Research
HQ Shellfish Research

Period Covered: March 1st, 2014 to September 30, 2014

Field Dates:
June 2013: red king crab collections in Bristol Bay, eastern Bering Sea
January 28-30, 2014: spot shrimp collections in Southeast Alaska
February/March and possibly June 2014: Tanner crab collections near Kodiak, Alaska

Plan Type: Category II

Approval

Title	Name	Signature	Date
Project leader	Joel Webb	Joel B Webb	7/11/14
Biometrician	Shareef Siddeek	Siddeek	7/11/14
Research Coordinator	Chris Siddon	Chris Siddon	7/11/14

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PURPOSE

Age information plays a pivotal role in understanding population dynamics and designing harvest strategies for exploited species for which data are available. This regional operational plan describes a pilot study to investigate and if possible develop the methods necessary for the application of a novel direct age determination technique for commercially important crab and shrimp stocks in Alaska for which direct aging has not been previously possible.

BACKGROUND

VALUE OF AGE INFORMATION IN FISHERY MANAGEMENT

Age data obtained from growth bands recorded in hard parts plays a fundamental role in fisheries science and provides critical information for fishery management and understanding of stock and fishery dynamics with millions of fish aged each year globally (Campana 2005). Growth and mortality are dominant life history characteristics controlling stock productivity that can be calculated directly from comprehensive age information. Age at sexual maturity, longevity, population age composition, and catch age composition provide critical information for stock assessment and the design of harvest regulations which seek to balance yield and maintain stock reproductive potential at levels which buffer against the probability of harvest-mediated declines in future abundance.

The inability to directly age crustaceans is an impediment to the assessment and management of crustacean fisheries in Alaska. Due to lack of information on individual age, length-based population models (e.g., Zheng et al. 1995) which integrate data on abundance, harvest, growth, and size-frequency distribution are used for the assessment and management of major crab stocks in Alaska (NPFMC 2013). However, the accuracy of these models may be compromised if the assumed growth or mortality rates are not representative of processes *in situ*. For several Alaskan crab stocks, reliable growth information is limited (e.g., Somerton et al. 2013) or growth information was collected during time periods in which conditions may not be representative of factors currently observed. For other crab stocks, stock-specific growth information is not available so similarity of growth parameters is assumed and growth models applied from stocks for which they have been estimated. In contrast, stock assessment and harvest strategies based on age-structured models implicitly account for variability in growth and mortality (and thus stock productivity) since these factors are directly accounted for in comprehensive size- and abundance-at-age data (Quinn and Deriso 1999).

Numerous crustacean fisheries in Alaska in both federal and state waters are also managed without length-based models. Management of all of these fisheries is guided by the need to provide all males an opportunity to participate in reproduction prior to vulnerability to harvest (Kruse 1993). The management framework for these stocks frequently considers the abundance of pre-recruits (individuals expected to grow to legal size over a defined time period) in the decisions to open/close a fishery and set the exploitation rate. In these cases, availability of reliable age data (and thus growth) would provide direct information on potential spatiotemporal variability in growth, implications for size-at-maturity (e.g., Orensanz et al. 2007, Siddon and Bednarski 2010), and size classes used to define pre-recruit abundance and by extension the exploitation rate.

METHODS OF AGE DETERMINATION

For many species of fish and invertebrates, age can be determined directly from growth bands recorded in hard parts including bones, scales and otoliths in fish (Campana 2001); and statoliths (Jackson 1994), shell sections (Abele et al. 2009), or other calcified structures for invertebrates. Direct determination of age from hard parts in crustaceans was assumed impossible due to complete loss (and replacement) of all calcified structures with molting (reviewed by Vogt 2012). Alternative indirect methods of determining age and growth in crustaceans include captive observations, tag-recapture experiments, accumulation of the metabolic pigment lipofuscin in neural tissue, and the widespread application of modal analysis of size-frequency distributions to define what are assumed to be year-classes or cohorts (Hartnoll 2001). The recent development of lipofuscin as an indicator of age has provided key insights into the role of age-structure in exploited crustacean stocks (e.g., Sheehy and Prior 2008) but can be constrained in application due to methodological limitations (Alláin et al. 2011), the necessity of ground-truthing physiological age with specimens of known chronological age or by indirect methods such as modal analysis (Sheehy et al. 1994), and the need to account for individual variation and temperature effects on lipofuscin accumulation rates (Sheehy and Bannister 2002).

DIRECT AGING OF CRUSTACEANS

Although it has been assumed that crustaceans lose all hard structures during the molt, it recently has been shown that hard structures exhibiting growth bands are present in lobsters, shrimps and crabs (Leland et al. 2011, Kilada et al. 2012) and that the gastric mill of at least one lobster and one crab species are retained through the molt (R. Kilada and J. Leland, pers. comm.). It was also demonstrated that growth band counts within the endocuticle region of eyestalks and/or the gastric mill corresponded well with indirect estimates of age rather than with the expected number of molting events. Retention of the banding layer within the endocuticle through the molt and seasonal rather than molt-related formation of the bands were confirmed respectively by calcein staining and holding through several molts for juvenile lobster and the increase in mean band count over a period of six months (and several molts) for juvenile snow crab (Kilada et al. 2012).

STUDY SPECIES AND CURRENT STATE OF KNOWLEDGE

Red king crab (*Paralithodes camtschaticus*), Tanner crab (*Chionoecetes bairdi*), and spot shrimp (*Pandalus platyceros*) were selected for inclusion this study with the following considerations: commercial importance and pressing management concerns, applicability of research results to closely-related species, proximity and availability of stocks for subsequent field and laboratory phases of aging research near crustacean research centers in Juneau and Kodiak, species previously investigated by Kilada et al. (2012) which included snow crab and shrimp, and availability of personnel to support research progress.

The level of information regarding life history parameters and probable size-age relationships is variable among the study species with most data available for red king crab followed by Tanner crab and spot shrimp.

Red king crab are able to molt throughout their lifespan; mature females molt annually while large, mature males may “skipmolt” for one or more years (McCaughran and Powell 1977). Estimates of red king crab size-at-age relationships have been based on laboratory or *in situ* holding and growth observations (Powell 1967), tag-recapture data (Weber 1974), analysis of size-frequency distributions (Loher et al. 2001), or derived using growth models (McCaughran and Powell 1977). However these techniques are generally acknowledged to have limited ability to accurately characterize size-at-age due to high variability in growth (Loher et al. 2001) and decreasing male molt probability with increasing size (Zheng et al. 1995), which obscure size-at-age relationships. Size-at-age relationships are also hypothesized to vary within and among red king crab stocks due to temperature-mediated differences in growth such that male and female age-at-maturity is likely to vary interannually within a stock (Loher et al. 2001, Stevens 1990) and by two to three years among stocks (Loher et al. 2001, Powell 1967). For example, in Bristol Bay females were estimated to mature at 8 to 9 years of age (Loher et al. 2001) with a reproductive lifespan of up to 10 years (Hoopes and Karinen 1972) while near Kodiak Island, based on tag-recapture and growth data, females reached maturity at 5 years of age but data were not provided on female longevity (Powell 1967).

Tanner crab are a congener of snow crab with substantially similar life history and reproductive biology including a terminal molt (Donaldson et al. 1981, Hilsinger 1976). Analysis of size-frequency distributions suggests that females complete the molt to maturity at which time they start carrying eggs at ages 4 to 6, while males likely terminally molt at 7 to 11 years post-settlement in the Gulf of Alaska (Donaldson et al. 1981). Temporal trends of decreasing male and female size-at-terminal molt have been observed in Bering Sea Tanner crab (Zheng 2008) resulting in management changes. Male size-at-maturity varies widely among discrete stocks with proximate geographic distributions in Southeast Alaska (Siddon and Bednarksi 2010). It is unknown whether varying size-age relationships are associated with these observations. Tag-recapture data suggests that male Tanner crab survive at least four years after the terminal molt but similar data are not available for females (K. Spallinger, pers. comm.).

Spot shrimp are a congener of northern pink shrimp (*Pandalus borealis*) in which banding has been found (Kilada et al. 2012). Little about the basic life history is known with certainty. Spot shrimp are protandric sequential hermaphrodites, reproducing as a male at smaller body size before transitioning to sexually mature females at larger body size. The Department of Fisheries and Ocean Canada assumes a four year life cycle, with only one spawning event as a female in their production model (Boutillier & Bond 2000), while a mark-recapture study in the central Gulf of Alaska estimated a maximum age of 8 (Kimker et al. 1996), and a length frequency analysis a maximum age of 10 years in the same area (Armstrong et al. 1995). Obtaining reliable age at size, and age at sexual transition, is of upmost importance in understanding the population dynamics of spot shrimp stocks.

RESEARCH PLAN

STUDY DESCRIPTION

This pilot study will be conducted by cooperative agreement with principal investigator Dr. Raouf Kilada at the University of New Brunswick. The study has a limited scope of investigation intended to guide and support future research if the objectives are successfully met. The primary objectives of this study are to determine if growth bands are observable and, if so, to develop and refine methods of application of this new aging technique to our species of interest.

A sample size of approximately thirty individuals per species (2 or 3 structures per individual) was based on the recommendation of the principal investigator as adequate for the objectives of this study within the study timeline (~ 7 mo.) and available budget. The sampling objective identified for each species was to provide individuals with a range of individual ages (and band counts) by providing samples distributed across the maximum available range of body size. For red king crab, females were selected due to annual molting rather than introducing the potential skip-molting phenomenon with males, which may further obscure size-age relationships (Powell 1967). For Tanner crab, males were selected due to the greater age and size range of males versus females prior to the terminal molt (Donaldson et al. 1981). Spot shrimp will include both males and females from small to large body size.

OBJECTIVES

Methods, deliverables, personnel/duties, and rationale are described for each objective.

1. Collect, dissect, and prepare the paired eyestalks and gastric mills of thirty red king and Tanner crab and the paired eyestalks of thirty spot shrimp for shipment to the University of New Brunswick.

   Red King Crab – PI: Joel Webb, Deliverable: 4/15/14 – The collection of female red king crab from Bristol Bay occurred in June of 2013 during the eastern Bering Sea National Oceanic and Atmospheric Administration Resource Assessment and Conservation Engineering Division crab and groundfish trawl survey. Twenty-seven females were haphazardly sampled from trawl collections across the available size range (~75-150 mm carapace length) to meet objectives by size bin. Sample location, date, and biological data were recorded for each individual. The crabs were frozen whole at the time of collection and shipped to Juneau by air freight. Sample preparation and shipping will be coordinated by Joel Webb.

   Tanner Crab – PI: Laura Stichert, Deliverable: 4/15/14 - The collection of male Tanner crab from Chiniak Bay (Kodiak) occurred in February – March, 2014 from the vessel *K-Hi-C* using cone pots. Males of pre-terminal molt status (adolescent; n=5) or new shell condition of post-terminal molt status (adult; n=55) were obtained across the available size range (~70-140 mm carapace width). Sample location, date, and biological data will be recorded for each specimen. The crabs were transported to the ADF&G Lab facilities at the Kodiak Fisheries Research Center in Kodiak the same day they were collected. If additional crabs are desired (within a smaller size range), collections will be obtained during the 2014 Westward Region trawl survey from the *R/V Resolution* on the eastside of Kodiak Island in June 2014. Sample selection, preparation, and shipping will be coordinated by Laura Stichert.

   Spot Shrimp – PI: Quinn Smith, Deliverable: 4/15/14 - The collection of spot shrimp from southeast Alaska waters occurred in January 2014 in concert with a planned sampling trip to capture live shrimp for research aboard the R/V Medea in Seymour Canal. Samples were collected to meet sample size objectives by length-bin across the range of body sizes (~20-67 mm carapace length) observed during sampling. Sample location, date, and biological data will be recorded for each specimen. Eyestalks were removed and preserved in the field. Sample collection was coordinated by Quinn Smith.

   Sample Preparation – Two eyestalks (and the gastric mill for crab) from each individual will be dissected, cleaned, and preserved in a solution of 60 % ethanol, 4 % glycerol, and 36 % water for at least three days. Prior to shipping structures to Dr. Kilada at the University of

New Brunswick, the preservative will be decanted and a cotton swab damp with the solution will be placed in the sample bottle for compliance with shipping dry samples under international air transport regulations.

2. Evaluate and confirm the presence or absence of growth bands in the eyestalks and gastric mill for red king and Tanner crabs and the eyestalks of spot shrimp and if present identify the structure, region within structure, and processing technique (mounting, sectioning, and imaging) optimal for reproducible age determination.

   PI: Kilada, Deliverable: As available but due by 8/31/14, Review: McNeel and Webb - Structures from each specimen will be mounted, thin-sectioned and examined for the presence of growth bands at the University of New Brunswick by the techniques described by Kilada et al. (2012). Application of the method to new species with varying morphology of the eyestalk and gastric mill will likely require extensive adjustment of techniques (e.g., section thickness and orientation) to identify optimal approaches. When processing of samples is complete for a species Kilada will provide to ADF&G a written description of the process necessary to refine techniques and a representative set of images showing the both the results of initial efforts and the refined approach. McNeel, Webb, and other ADF&G project leaders as available will confer with Kilada to review progress, challenges, and preliminary results for each structure and species. These meetings will be organized on a bimonthly schedule by Webb during the project period or more frequently if needed.

3. Confirm the presence and relative locations of cuticle layers (epicuticle, exocuticle, and endocuticle) within the eyestalk and gastric mill structures for red king and Tanner crabs and the eyestalk for all three species by histological staining.

   PI: Stichert, Deliverable: 6/1/14 - Confirmation of the location of the endocuticle within the cuticle structure is necessary for interpretation of the growth bands (Kilada et al. 2012). Histological evaluation of each structure from each species (eyestalks from all species and gastric mill from crab species) will be coordinated by ADF&G project personnel to confirm the location of three cuticle layers (epicuticle, exocuticle, and endocuticle). To ensure we obtain a high quality image of each structures of interest, a sample size of 3 individuals will be collected for each of the species for a total of n=15 structures. Samples will be cleaned and preserved in Bouin's solution for at least a week then transported to the ADF&G Pathology Laboratory. Structures will be processed (prepped and embedded in paraffin) and cross sections will be obtained at or near the location of interest for growth bands and stained following Masson's Trichrome Stain procedures. Stained and mounted cross sections will be imaged as a reference for the location of cuticle layers for each structure and species combination.

4. Contingent on objective 3, obtain band counts for each structure and compare band counts among structures (2 eyestalks and 1 gastric mill for crabs and 2 eyestalks for shrimp) within the same individual and between two independent readers for each structure and species.

   PI: Kilada, Deliverable: As available with all products due by 8/30/14. Review: McNeel and Webb – For each structure from which an age estimate was obtained Kilada will deliver to ADF&G an image library referenced to the sample and annotated with identified bands and age estimate. Kilada will also prepare bias plots (Campana et al. 2001, and see Figure S7 in Kilada et al. 2012 for example) which are a standard method of quantitative evaluation of the level of agreement of age estimates among structures within specimen and between two independent readers. McNeel, Kilada, and Webb will evaluate results for evidence of bias or systematic error.

5. Contingent on objectives 3 and 4, comparison of band-based estimates of individual age with information available on size-at-age for each species from peer-reviewed or technical literature.

   PI: Kilada with support from ADF&G project leaders coordinated by Webb, Deliverable: with final report due on 9/30/14 - Text and graphical description and explanation of band-based age estimates compared to published size-age relationships.

6. Final report on project results and conclusions in relation to each objective.

   PI: Kilada, Deliverable: Final project report due to ADF&G on 9/30/14, Review: ADF&G project leaders with final approval by ADF&G Chief Marine Fisheries Scientist. The final report will provide analysis and results in relation to each project objective and conclusions and recommendation regarding the applicability of the band-based aging method to each species. The project leaders and Kilada will collaborate to determine as the research progresses whether the final report will be formatted as a peer-reviewed manuscript.

PLANNING FOR FUTURE RESEARCH

In preparation for this pilot study, the project leaders, ADF&G fish and invertebrate aging staff, and Dr. Bodil Bluhm from the University of Alaska Fairbanks met with Dr. Kilada in December of 2013 to receive training in the collection and preservation of crab and shrimp eyestalks and crab gastric mills. Structures from one individual of each species were processed (sectioned, mounted, polished) at the ADF&G Mark, Tag, and Age laboratory in Juneau and examined for the presence of growth bands. This cooperative investigation provided preliminary positive results of the presence of visible banding in the gastric mill and eyestalks of red king crab (Figure 1a-1b), the eyestalk of Tanner crab (Figure 1c), and the eyestalk of spot shrimp (Figure 1d) from Alaska.

Beyond the initial steps of evaluation and development of methods for the application of this aging technique to important crustaceans in Alaska for which this pilot study is designed, crustacean age determination research in Alaska will likely be a multi-faceted program involving multiple agencies, species, and research objectives. To facilitate development of this planning process the project leaders met with Dr. Bob Foy, Director of the National Marine Fisheries Service, Kodiak Laboratory for discussion of near-term and long-term research objectives, resources, and points of collaboration. Outcomes of this meeting (summarized in Appendix 1) were 1) to encourage broad and open collaboration; 2) to identify interested researchers within ADF&G, NMFS, and the University of Alaska; 3) to prepare and disseminate a draft plan to help guide crustacean age determination research (Foy); and 4) to ascertain near-term research objectives, the foremost of which are technique development and a workshop training opportunity for aging experts from ADF&G, NMFS, etc and studies on preservation effects on structures used for aging.

BUDGET

Funding for the ADF&G components of this pilot study excluding the histology objective and limited shipping costs will be covered under existing personnel and supply budgets. The UNB project component will funded through a cooperative agreement between the University of New Brunswick and the Alaska Department of Fish and Game. Funds will be distributed by cooperative agreement with 90% of funding disbursed on the project start date and 10% on approval of final report.

Proposed Funding for University of New Brunswick project component by cooperative agreement:

				Subtotals:
1A.	WAGES[a]			
		Hourly Rate	Total	
	a. Dr. Raouf Kilada	$41.17	$24,500.00	$32,500.00
	b. Laboratory Technician	$23.52	$8,000.00	
1B.	FRINGE BENEFITS			
		Rate	Total	
	a. Dr. Raouf Kilada	18.25%	$4,471.25	$5,751.25
	b. Laboratory Technician	16%	$1,280.00	
1C.	SUPPLIES (for 240 Samples)			$2,000.00
1D.	SUBTOTAL DIRECT COSTS			
1E.	INDIRECT COSTS @ 0%			$0.00

[a] Based on an estimated 600 hours for Dr. Kilada and 340 hours for technician

UNB TOTAL $40,251.00

Proposed Funding for ADF&G project component:

1.	SUPPLIES AND SHIPPING	$500.00

ADF&G TOTAL $500.00

TOTAL PROJECT FUNDS REQUESTED **$40,751.25**

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FIGURES

(a) (b) (c) (d)

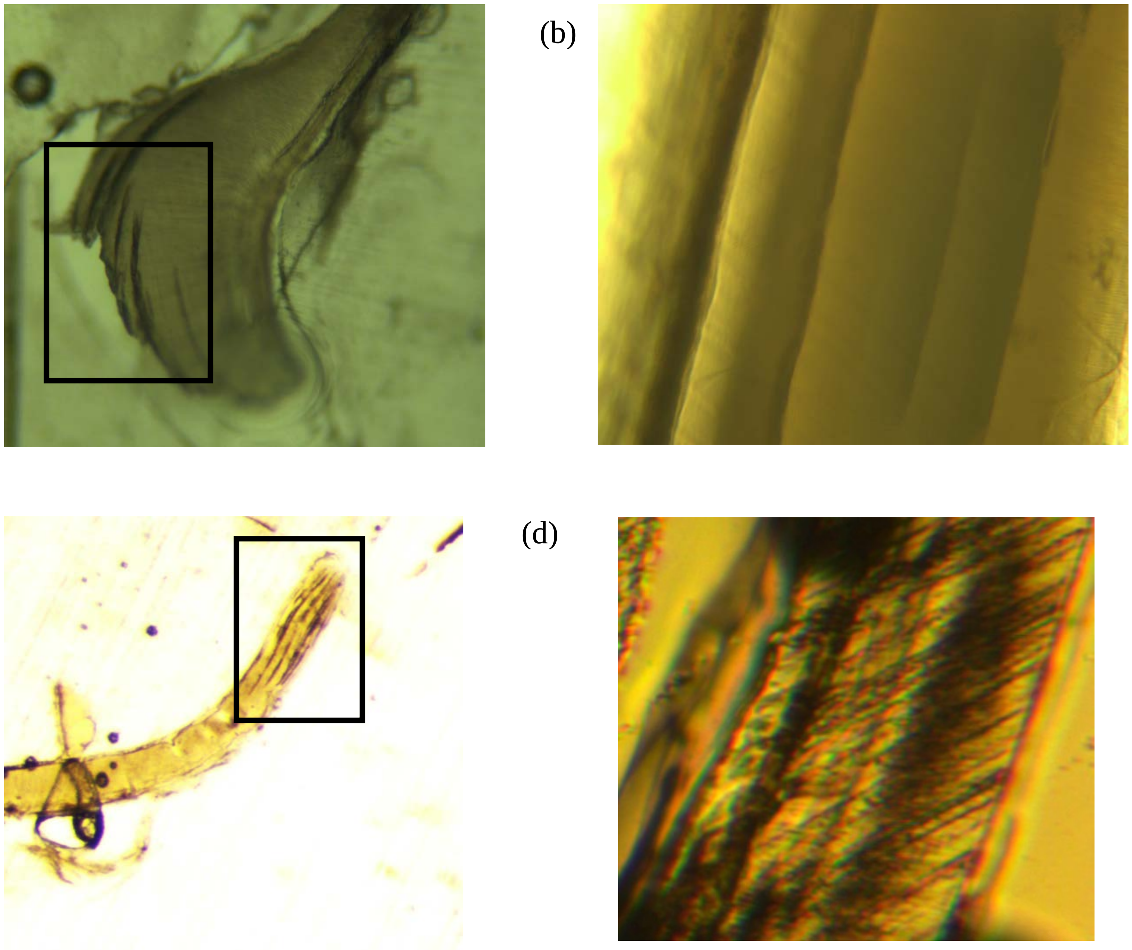

Figure 1.–Banding, potentially indicating age, in the a) gastric mill of red king crab, b) eyestalk of red king crab, c) eyestalk of Tanner crab and d) eyestalk of spot shrimp. Boxes indicate region of banding within the structure in a and c.

APPENDICES

Appendix 1.–Summary of crustacean age determination research planning meeting

Crustacean age determination planning meeting – February 5, 2014

Participants: Bob Foy, Kevin McNeel, Quinn Smith, Laura Stichert, and Joel Webb

Objective: Identify areas of cooperation and collaboration to advance research on application of new direct aging methods to crabs and shrimp in Alaska

1. Resources available for Research:

- ADFG:
    - People: Joel, Laura, Quinn, and Kevin available at varying levels with Joel having the most flexibility for specific support.
    - Resources to Support Lab and Field Research:
        - Small and large research vessels and dive team in Southeast Alaska
        - Aging Determination Unit (ADU) currently working on fish and invertebrates in Juneau. ADU will participate in development, training, and future studies with greater availability likely in 2015 v. 2014.
        - State-waters crab surveys for Tanner and red king crab
        - Saltwater holding and laboratory in Kodiak for laboratory-based age experiments.

- NMFS:
    - People: Bob and potentially patho-bio group (Pam, Christy, Vanessa) and/or post-doc as research progresses
    - Resources to Support Lab and Field Research
        - Divers for sample collection and potential field experiments
        - Age determination laboratory in Seattle
        - Ability and expertise to use cultured known age individuals of red and blue king crab in laboratory experiments (e.g., calcein marking).
        - Interest in using calcein as a retainable marker for releases of cultured juveniles
        - Varying numbers of frozen and live laboratory-reared individuals of known age for RKC, BKC, and TC

2. Current Research Directions:
    - ADFG: Is collaborating with Dr. Kilada on a pilot study to develop and refine the application of band-based aging in three Alaskan species the spot shrimp, Tanner crab, and red king crab. Study objectives are to identify the best structure (gastric mill versus eyestalk) and site within structure to perform band counts. Species-specific development is necessary due to morphological variation in structures among species and application of the technique to species that have not previously been investigated. Further project objectives will be to compare band-based age estimates across the maximum possible body size range to corroborate estimates

relative to the best available information and perform histological examination to confirm cuticle structure for each species.

- NMFS: Has grant to initiate culture of snow crab from egg to benthic stages with potential to create a collection of known age crab for validation of aging method and calcein marking experiments. Early benthic stage juvenile blue and red king crab are currently being held in the lab and could be used for calcein marking experiments or other age-related research. Progress will depend on the result of an internal request for funds.

3. Points for Collaboration:
    - The highest priority is to build capacity to conduct aging work in Alaska. A training workshop led by Raouf Kilada to train aging personnel from both NMFS and ADFG in techniques for aging crustaceans is a near-term opportunity that should be a focus of collaboration. NPRB workshop funding in the RFP may be an avenue for this effort. Dr. Kilada's feedback was that a productive workshop would best occur following the pilot study of method development for Alaskan species.
    - The NMFS Kodiak Laboratory has samples of known-age animals for RKC, BKC, and TC. Blind reading of these specimens for age is a high-priority and should be included in the first phases of age determination research.
    - A preservation effects study e.g., freezing vs live dissection is needed since many of the existing available samples are frozen.
    - Bob will develop and distribute a "Crustacean Age Research Plan" as a focal point for aligning research directions and organizing collaborations on age determination research among agencies.
4. Outcomes:
    - Joel will summarize the meeting results and distribute for review.
    - Bob will draft the "high level, forward looking" research plan and distribute for review in the coming month.
    - Joel will contact Sherry Tamone as a possible university contact for interest in aging work and Bodil Bluhm for any suggestions regarding other collaborators within UA.